Fast Human Phospho-Tau Thr217 ELISA

Catalog Number:EK20417

For the quantitative determination of human pTau-217 concentrations in cell culture supernates, serum, and plasma.

This instruction must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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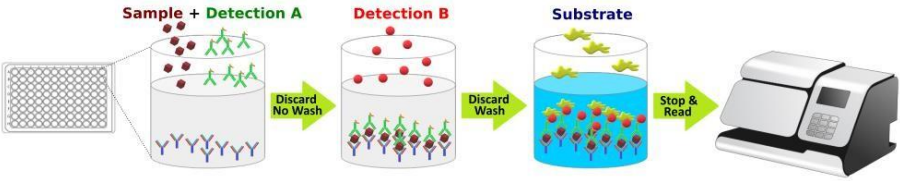
INTRODUCTION

Phospho-tau217 (p-Tau217) is one of the most robust plasma biomarkers for Alzheimer’s disease. In many studies, the marker identified people with the highest likelihood of Alzheimer’s disease, almost 100 percent accuracy. In differential diagnosis, plasma p-tau217 also distinguished AD from other neurodegenerative diseases, notably including tauopathies, with high accuracy, beating out other plasma markers including neurofilament light and p-tau181. Tribioscience’s Fast Human Phospho Tau Thr217 (p-Tau217) ELISA is designed to quantitatively detect human p- Tau217 levels in serum, plasma, and other biological samples. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The Hands-on time can be within 2 hours, not need 4-5 hours (Fig. 1). **The detection range is from 0.3 to 243 ng/mL**. The levels of human p-Tau217 samples are parallel to the standard curves obtained using the kit standards linearly. Therefore, the kit can be used to determine relative mass values for natural human p-Tau217 protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for human p-Tau217 was pre-coated onto a microplate. Standards or samples and Detection Antibody are pipetted into the wells, and concurrently incubated for 2 hours. Then, just aspirate each well, no wash, directly add Secondary Antibody, incubate the complex. Following a wash to remove any unbound antibody andsamples, an ultra- sensitive TMB substrate solution is added to the wells for color develops. The color intensity is in proportion to the amount of bound in the initial step. The intensity of the color is measured by plate read at 450 nm.



KIT CONTENT AND STORAGE CONDITIONS

PART	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human PTau-217 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human p-tau217.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Human PTau-217 Standard	50 µl of Recombinant human p-tau217 (2.43 µg/mL).	Aliquot and store at -20 ℃ for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	2.1 ml of human p-tau217 Detection antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	12 ml of HRP conjugated secondary antibody.	
Assay Diluent	12 ml of a buffered protein base with preservatives.	
Wash Buffer	12 ml of concentratedsolution(10x).	
TMB Substrate	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	6 ml of 2 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date. The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

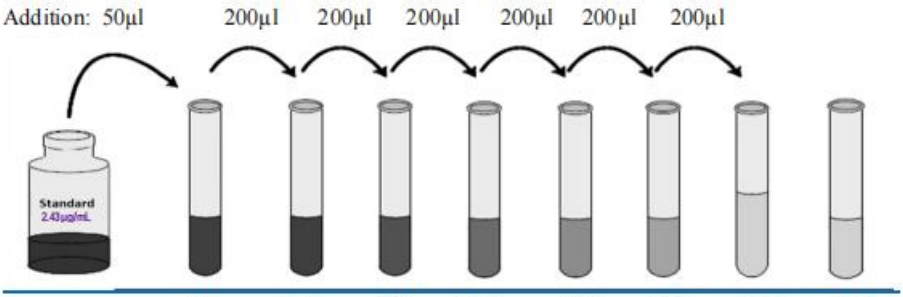
Wash Buff er: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved).

Human p-tau217 Standard Preparation: Label test tubes as #1 through #8. Pipet 450 µL of 1x Assay Diluent into tube #1, and 400 µL into tubes #2 to #8 as diagram below.

1. Add 50 µL of the Human p-tau217 Standard stock solution (2.43 µg/mL) to tube #1 and mix.

2. Make 3x serial dilutions of the standard using the Tube#1(243 ng/mL standard solution) from Tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 243, 81, 27, 9, 3, 1 and 0.33 ng/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Human pTau-217 standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	450	400	400	400	400	400	400	400
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	50	200	200	200	200	200	200	0
Final Conc (ng/mL)	243	81	27	9	3	1	0.33	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at RT for 1 hour.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of Detection B to each well. Incubate at RT for 30 min.
5. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (Complete removal of liquid at each step is essential to good performance). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **TMB Substrate** to each well. Incubate at RT for 10-20min (Protect from light). The color becomes blue.
7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).

8. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Averagethe duplicate readings for each standard, control, and sample subtract the averagezero standard optical density (O.D.). Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

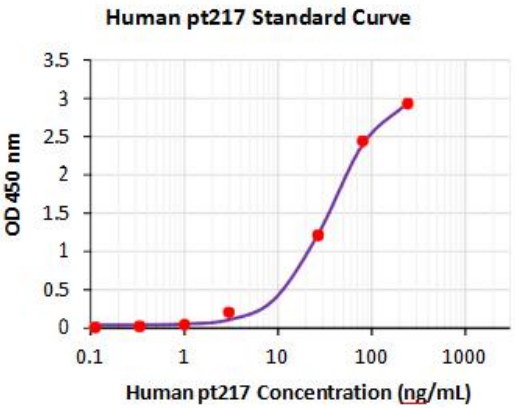
This standard curve(R2=1.000) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

The minimum detectable dose (MOD) of human is typically 200 pg/ml. The Intra-assay CV and the Inter-assay CV are <10%.

SPECIFICITY

This assay recognizes natural and recombinant human pTau-217.



No cross-reactivity with others.